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AN AUTOMATED HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC CLEANUP PROCEDURE FOR THE DETERMINATION OF CHLORINATED DIBENZO-P-DIOXINS AND CHLORINATED DIBENZOFURANS

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Introduction

The need for detection of ultra-trace levels of chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs) has become a priority in environmental labs because of the possible human health concerns of the 2,3,7,8-chlorine substituted dioxin and furan congeners. CDDs and CDFs occur as byproducts in the manufacturing and combustion of various chlorinated compounds such as chlorinated phenols (eg. pentachlorophenol, which was used on a large scale in fungicides and wood preservatives), phenoxy herbicides, chlorinated diphenyl ethers, and polychlorinated biphenyls(1). The introduction of these pollutants into our waterways from industrial sources is evident when analyzing various biota samples.

CDDs and CDFs bioaccumulate readily in animal fat tissue because of their lipophilic nature and stable physical properties(2). Other chlorinated organic compounds are present in many samples at concentrations several orders of magnitude higher than those for CDDs and CDFs. To obtain acceptable detection levels, this particular analysis requires very sensitive and specific analytical techniques. After extraction of the analytes from the matrix, the bulk of the interferences are removed with an initial cleanup of the sample extract. An important class of interferences are chlorinated diphenyl ethers(CDPEs)(3)(4). These compounds fragment under electron ionization conditions in the mass spectrometer ion source to form ions isobaric with CDF molecular ions, producing false positive results. Low-resolution, high-resolution, or tandem mass spectrometers (LRMS, HRMS, MS-MS) cannot distinguish the CDFs from the CDPEs. Therefore the CDPEs must be removed prior to the GC/MS analysis. The Ontario Ministry of Environment (MOE) has developed a sample preparation procedure incorporating an automated dual column HPLC cleanup step to address this specific interference and this development allows the sample to be analyzed by LRMS techniques.

Experimental Section

Sample Preparation

Twenty grams of ground and homogenized fish tissue was weighed into an Erlenmeyer flask. Each sample was spiked in the flask with a surrogate standard solution containing five 2,3,7,8 substituted $^{13}C_{12}$-labelled dioxin isomers, one from each congener group. The sample was then digested overnight (approximately 16 hours) in concentrated hydrochloric acid. The acid digested tissue was extracted with hexane and the extract was passed through a cylindrical funnel packed with anhydrous sodium sulphate and sulphuric acid impregnated silicic acid. The extract was concentrated and quantitatively transferred to a 100 uL conical vial and taken to dryness. The sample was then reconstituted in 150 uL of hexane and was ready for injection onto the HPLC system.

Instrumentation

The automated HPLC cleanup procedure involves the use of a column-switching step whereby the CDD and CDF fraction is trace-enriched from a neutral alumina HPLC column onto another HPLC column packed with a carbon-silica mixture. The CDDs and CDFs are retained on the carbon-silica column while interferences such as polychlorinated biphenyls, polychlorinated diphenyl ethers, and some polychlorinated napthalenes are not retained. The CDDs and CDFs are recovered from the carbon-silica column by backflushing the column with toluene. The automated HPLC system consists of the following components, all of which are computer-controlled:

-Automated sample processor and injector
  - Gilson model 232-401
-HPLC pumps (3) - Gilson model 302 piston pumps
  - only 2 are used for forward elution
  - solvent used is hexane and dichloromethane
-Programmable HPLC pump
  - Gilson Model 305
  - solvent used is toluene
-Ultraviolet Detector
  - Gilson Model 116 - programmable
  - Fraction collector
  - Gilson Model 202
-Dynamic Mixer - Gilson Model 811B
-Manometric module - Gilson model 802C
-6-port switching valves (2)
-HPLC columns (2) - Normal Phase Alumina, (0.46 cm ID x 25.0cm from Phenomenex, Torrance, CA, USA), Spherisorb with 5 micron particles

- Empty Waters guard column (0.46 cm x 3.0 cm) packed in-house with a mixture of 5%(w/w) Amoco PX-21 carbon/ silicic 70-230 mesh)

After HPLC cleanup, the samples were concentrated and quantitatively transferred to 100uL conical vials. The samples were then ready to be analyzed by the following gas chromatography/mass spectrometry techniques.

Gas Chromatography Low Resolution Mass Spectrometry (GC/MS)

Fifteen fish samples were analyzed using a Finnigan 4500 GC/MS. Samples were introduced onto a 30m DB-5 fused silica capillary column(0.25mm I.D. with 0.25μm film thickness) via an on column injector at ambient temperature. The GC conditions were as described previously(5). The GC effluent was directly interfaced to the mass spectrometer. The instrument was tuned using perfluorotributylamine(PFTBA).

CDD's and CDF's were detected using selected ion monitoring (SIM) techniques. The ions monitored for the native CDDs/CDFs correspond to the three most abundant molecular ions. Only two ions are monitored for the $^{13}C_{12}$-labelled surrogate standards. Two ions are also monitored with each congener group for the CDPEs.

Gas Chromatography Tandem Mass Spectrometry (GC/MS/MS)

Twenty five fish samples were analyzed using a Finnigan TSQ70 GC/MS/MS. The samples were injected on a 60m DB-5 column (0.25mm I.D. with 0.25 μm film thickness), through a splitless injector, at 300°C. The GC was programmed as follows: hold initial column temperature at 120°C for 1 minute, ramped at 7.5°C/min. to 250°C, then ramped to 300°C at 2.5°C/min., and hold for 10 minutes. The carrier gas was helium, at a column head pressure of approximately 22 PSI. As previously described(6), the instrument was tuned using CDDs.

The interferences which affect LRMS sensitivity and selectivity are those compounds which are co-extracted with CDDs and CDFs and are not removed in the cleanup steps. MS/MS can be used to distinguish CDDs and CDFs from chemical interferences by selected reaction monitoring (SRM) techniques. MS/MS detection consists of the mass selection of a parent ion in the first mass analyzer, fragmentation of this ion by collision induced dissociation(CID) and final detection of the daughter ion in the second mass analyzer. Interferences such as CDPEs can not be distinguished from CDFs by MS/MS or any other type of mass spectrometry because under EI conditions they fragment to form ions isobaric with CDF molecular ions in the ion source.

Results and Discussion

The automated HPLC dual column cleanup method developed at MOE was found to be very effective in removing interferences such as PCBs and CDPEs. The recoveries of the internal standards were found to be acceptable and consistent, with a 62% average recovery. The extract was clean enough to allow the analysis to be carried out by LRMS techniques. The detection limits achieved by the Finnigan 4500(LRMS) were typically 4 to 5 PPT. Although these were good detection limits, a detection limit of 4 PPT is required to obtain "Toxic equivalent" detection limits below the levels required for a warning to be issued in the MOE/MNR "Guide to Eating Ontario Sports Fish". The sensitivity of MS/MS techniques was preferred because the detection limits achieved by LRMS were too close to the guideline. The detection limits obtained by MS/MS analysis were typically less than 3 PPT.

Further development will test the applicability of this cleanup procedure to other environmental matrices where CDPEs and other organic interferences are problems. Future work will also include the determination of the active lifetime of a carbon column and optimize the column for maximize lifetime.

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